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Patterns and Processes of Larval Emergence in an Estuarine Parasite System

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Abstract. Trematode parasites in intertidal estuaries experience constantly varying conditions, with the presence or absence of water potentially limiting larval transport between hosts. Given the short life spans (≤24 h) of cercariae, emergence timing should be optimized to enhance the probability of successful transmission. In the present study, field measurements and laboratory experiments identified processes that regulate the emergence of cercariae from their first intermediate snail hosts in an intertidal marsh. Larvae emerged over species-specific temperature ranges, exclusively during daylight hours, and only when snails were submerged. The three factors operate over different temporal scales: temperature monthly, light diurnally (24-h period), and water depth tidally (12-h period). Each stimulus creates a necessary condition for the next, forming a hierarchy of environmental cues. Emergence as the tide floods would favor transport within the estuary, and light may trigger direct (downward or upward) swimming toward host habitats. Abbreviated dispersal would retain asexually reproduced cercariae within the marsh, and local mixing would diversify the gene pool of larvae encysting on subsequent hosts. In contrast to the timing of cercarial release, emergence duration was under endogenous control. Duration of emergence decreased from sunrise to sunset, perhaps in response to the diminishing lighted interval as the day progresses. Circadian rhythms that control cercarial emergence of freshwater species (including schistosomes) are often set by the activity patterns of subsequent hosts. In this estuary, however, the synchronizing agent is the tides. Together, exogenous and endogenous factors control emergence of trematode cercariae, mitigating the vagaries of an intertidal environment.

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Introduction

Parasite larvae typically disperse prior to finding and infecting a host. As with propagules of free-living organisms, such as crabs (Forward *et al.,* 1986; Morgan, 1996), sponges (Amano, 1988), and plants (Horn *et al.,* 2001; Schauber *et al.,* 2002), external cues may direct emergence of parasite larvae under favorable conditions. Because dispersal stages of digenetic trematodes have life spans of 24 h or less (McCarthy, 1999; Toledo *et al.,* 1999), timing emergence to correspond with host availability would be especially advantageous (Combes *et al.,* 1994; Pechenik and Fried, 1995). Moreover, the widespread distribution of trematodes in fresh water (Pages and Theron, 1990; Gerard, 2001) and saltwater (Martin, 1972; Bartoli and Combes, 1986; Jonsson and Andre, 1992; Curtis, 1997) environments allows for cross-habitat comparisons of emergence characteristics.

Trematode emergence has been studied largely in freshwater systems, with much of this research addressing medical and agricultural concerns (Bergquist, 2002; McKerrow and Salter, 2002). In common parasites, such as schistosomes, larval emergence from intermediate host snails varies on a circadian cycle and is synchronized with definitive host availability (Pages and Theron, 1990; N'Goran *et al.,* 1997). Circadian rhythms are usually entrained by photoperiod or thermoperiod (Theron, 1984; Mouchet *et al.,* 1992; Combes *et al.,* 1994). Freshwater parasite larvae moving from aquatic to terrestrial vertebrate hosts time their emergence to coincide with waterfront activities of the hosts, on scales of hours (Theron, 1989; Raymond and Probert, 1991).

In southern California intertidal marshes, there is a guild of more than 18 digenetic trematode species (Martin, 1972). Sexual reproduction occurs in definitive shorebird hosts, which defecate parasite embryos into the marsh. Free-living miracidia hatch and infect the California horn snail, *Cerithidea californica* (Haldeman), causing castration and other sublethal effects (Sousa, 1983; Sousa and Gleason, 1989). Asexual reproduction ensues, producing tens to thousands of cercariae per snail per day. The cercariae are produced in the area previously filled by the snail gonad, and the larvae then crawl within the snail hindgut to emerge from tissues in the rectum. Once released into the environment, cercariae encyst on second intermediate hosts, such as benthic snails (including *C. californica*), crabs, and fishes. Ingestion of these intermediate hosts by birds completes the parasite life cycle. Swimming cercariae are short-lived, so they must move toward or remain near host habitat for effective transmission. In marshes, tidally varying water depth and currents may limit transport.

The purpose of the present study was to identify factors controlling cercarial emergence in an intertidal estuary, as compared to freshwater systems. Two hypotheses were tested. (1) Timing of cercarial release varies over the day, as in freshwater trematodes. Light (24-h period) may impose a similar diel periodicity in both systems, but tidal (12-h period) effects (presence or absence of water, currents) would be unique to the estuary. (2) Exogenous factors control cercarial emergence in intertidal estuarine habitats, whereas in many freshwater species, larval release is controlled endogenously. Circadian rhythms in freshwater cercariae are often associated with innate activity patterns of intermediate or definitive hosts (Combes *et al.*, 1994). Such predictable host signals may not be important in an estuarine system, where submergence is limited by the tidal cycle.

Our research was conducted in two stages. Field studies measured emerging parasite larvae as a function of external variables (temperature, salinity, tidal height). Then, laboratory experiments identified the role of the specific factors in controlling the onset and duration of emergence. We herein define "emergence" as the shedding of cercariae from intermediate host snails. This study does not distinguish between the effects of larval behavior and rates of asexual reproduction on cercarial emergence patterns.

Materials and Methods

Field observations

In the field, we collected emerging cercariae of all species from snails (*Cerithidea californica*) while simultaneously measuring environmental variables. Data were collected on 38 days from 1999 to 2002. Measurement intervals were selected to span all hours of the day and months of the year. Moreover, six night collections ($\geq$ 1 h after sundown) were paired with a day collection on the preceding or proceeding tide to compare emergence within the same group of snails.

The field site (tidal channel) was located in Carpinteria Salt Marsh Reserve (CSMR), east of Santa Barbara, California (34°24′16″ N, 119°31′30″ W). Detailed physical characteristics are given in Fingerut *et al.* (2003). Biological measurements took place along the centerline of a channel 400 m long, 5 m wide, and 0.6 m deep (at high tide). A centrally located 3-m-wide mudflat was carpeted (460 $\pm$ 16/m^2) with *C. californica*, the first intermediate host for the trematodes studied here. The snail population extended hundreds of meters in the along-channel direction. The hydrodynamic regime in this and similar southern California marshes (*e.g.*, Mission Bay and Newport Bay) was dominated by slow flows ($<$ 5 cm/s), with shear velocities ($u*$) of 0.02 cm/s or less occurring more than 80% of the time (Fingerut *et al.*, 2003). Rarer, storm-driven currents ($u* \geq$ 0.8 cm/s) occurred only about 1% of the time. Intertidal mudflats in channels were located 1.3 m above MLLW (mean lower low water) and were inundated twice a day by the semidiurnal tide. Water depth exceeded this height for an average of 4 h during each 12-h tidal cycle. Daily variations in salinity were 28–34 psu, consistent across seasons (Fingerut *et al.*, 2003). Salinity and temperature were relatively constant throughout the water column, indicating well-mixed conditions in the shallow channel.

Collections of emerged cercariae. A specially designed larval collector was used to measure (non-intrusively) cercarial emergence from a specified group of snail hosts. The main body of the collector was a flat, clear acrylic chamber (50 cm wide by 50 cm deep by 2.5 cm tall) with 34-μm-mesh side panels and a solid bottom. Penetrating the top of the chamber was a 4 by 4 array of 8-cm-diameter holes (covering 32% of the surface area), each fitted with an inverted funnel (*i.e.*, small end pointing away from the chamber) intake. Contents of all funnels were united into a single 12.5-mm (ID) tube that fed into a peristaltic pump (Masterflex IP variable speed). The pump continuously filtered water from the chamber at a rate of about 5 liters/min over a 34-μm mesh. The chamber volume was flushed 8 times during each 10-min sampling interval.

During each sampling event, 200 snails—from the largest ($\approx$2.5-cm-long) size class in the marsh—were placed in the collector. Once on the mudflat, the chamber was gradually inundated by the incoming tide at the same time as the free-ranging snail population. There was no movement of cercariae into or out of the chamber. Captives were provided with oxygenated water at ambient temperature. Thus, cercariae collected on the filter could be ascribed solely to the enclosed host population. Size ranges of marsh cercariae are 50–100 μm (width) and 200–1000 μm (length) (Martin, 1950; Adams and Martin, 1963), so the mesh retained all species.

The system self-primed when the water level reached the

bottom of the funnels (5 cm water depth), usually 5–10 min after the tide first rose to the level of the mudflat. Every 10 min the filter was removed (and replaced), dipped in 90% ethyl alcohol, and placed in a sealed petri dish for later staining (0.5% Lugol's solution) and counting. Samples were also examined to identify numerically dominant species throughout the year and day. Each collection lasted 4 h, matching the average period of snail inundation. A cumulative frequency distribution was assembled from the total number of emerged cercariae during each 10-min collection. The time for 95% of all cercariae to emerge was compared between collections.

Environmental measurements. Properties of the physical environment during tidal inundation (4-h interval during flood or ebb) were measured on the 38 periods of quantified cercarial emergence. The mudflat was exposed to air for about 8 h between each tidal inundation. Maximal water depth was 60 cm during each tide. Using an ORION model 140 probe, seawater temperature and salinity were recorded 1 cm above the bed every 10 min. An underwater quantum sensor (LICOR model 190SA) was flush-mounted at the mudflat surface before tidal inundation to register light intensity at 1 Hz over the duration of the flood and ebb tides.

Laboratory experiments

Field measurements (see *Results*) indicated that the following factors may control cercarial emergence: temperature, host inundation (water depth), light, and time of day. Because we used only infected snails (rather than a random sample of the host population) in laboratory experiments, numbers of cercariae emerging were 10–100 times higher than those reported for field observations. With one exception, all laboratory experiments were conducted during summer months (June–September). Trials to evaluate cercarial emergence as a function of water temperature were performed during the spring (March–April).

Effects of temperature. Two time series were conducted in the laboratory, each using the same 53 snails (*C. californica*). The animals were placed individually in chambers (27 cm^3) filled with seawater. In the first 7-day series, snails were held at a higher (+1 °C) water temperature on consecutive days, over the range 13–19 °C. Water was changed to the new temperature at 1000 h each day, and cercariae were collected 4 h later. (All times are reported as Pacific daylight time). Snails were kept dry at a constant 18 °C between runs (average spring air temperature for daylight hours). The second 7-day series followed the same protocol, except that exposure temperature was decreased 1°C per day, from 19 to 13 °C. At the end of a 4-h incubation period, contents of individual chambers were processed separately to determine species (Martin, 1972) and number of emerged cercariae.

Effects of host inundation. Two groups of 100 snails each were randomly assigned to one of two treatments, and were placed in trays (21 cm long by 11 cm wide by 3 cm deep), at 20 snails per tray. Both groups were then situated in an environmental chamber at 21°C (average summer water temperature) with constant light (40 μmol/m^2/s). For one group, trays were kept dry for 4 h; for the other group, trays were filled with water from CSMR. At the end of 4 h the dry trays were briefly filled to suspend any emerged cercariae, and the water from both groups was filtered through a 34-μm mesh. The number of emerged cercariae from each treatment was determined using the same processing methods as for the field samples. The following day, the treatments were reversed for the two snail groups. This 2-day experiment (one 4-h test period each day) was replicated three times, with a new batch of snails each time.

Effects of light intensity. Snails were exposed to a midday intensity (~2000 μmol/m^2/s) or to simulated dawn/dusk intensity (~40 μmol/m^2/s), created by reducing midday intensity with a neutral density filter. Snails (100 per group) placed in water-filled trays (see *Effects of host inundation*) were randomly assigned to one of the light conditions. Every 15 min, trays were overturned onto a 1-mm-mesh panel. Snails remained in the trays, but water and cercariae passed through. Trays were immediately refilled. Water and larvae were filtered over 34-μm mesh and processed as in the field study. To control for the warming effect of the stronger sunlight, snail trays were held in a water bath maintained at 21°C (average for summer months). Trials lasted 2 d, with each group exposed to both conditions on consecutive days, and was replicated three times, each with different snails.

Effects of time of day. Two series of experiments were performed, one testing time of day (TOD) on emergence duration and the other testing for the existence of an endogenous rhythm. In the first series, 100 snails were placed in water-filled trays (see *Effects of host inundation*) at constant temperature (21°C) and light (40 μmol/m^2/s) for 4 h, starting at a different time each day (0900, 1200, 1500, and 1800). During each 4-h interval, emergence duration was determined as in the light experiments. Three replicate trials, each with new snails, were run at all four start times.

The goal of the second series of experiments was to determine if an endogenous rhythm, stimulated by light, might control emergence duration. This research is necessary but not sufficient to establish a circadian rhythm (Aschoff, 1960; Pittendrigh, 1993; Dunlap, 1999). The experiment held light:dark ratio constant while varying the onset of daylight. The prediction was that emergence duration would track with an internal clock set by incipient dawn, independent of the absolute time of day.

Two temperature- and light-controlled chambers were set at 21°C and 40 μmol/m^2/s. The control chamber was set on a light:dark (14:10) cycle, with the natural (0600) sunrise. The experimental chamber used the same light:dark ratio

but with sunrise shifted either 8 h forward to 1400 or backward to 2200. In the first experiment, 100 snails were placed in the control and another 100 in the forward-shifted light chamber for 1 week before the trial began. After this acclimation period, emerging cercariae in both chambers were monitored over 4 h, beginning at 1800, as in the light experiments. In the second experiment, new snails were acclimated to the control and backward-shifted light regime for 1 week, and snails were monitored for 4 h beginning at 1000. In both experiments, the control and light-shifted snails were tested on sequential days. Moreover, as a check on the repeatability of the results, the same set of snails was monitored over an additional 4 d. Both experimental series were conducted once in 2000 and once in 2001.

Results

Field observations

The magnitude of cercarial emergence correlated significantly with water temperature, but not with salinity or total irradiance (Table 1 and Fig. 1). Snails (*Cerithidea californica*) first appeared in tidal channels in February, when temperatures rarely exceeded 13 °C (Fig. 2A). Emergence did not begin until March, however, when seawater warmed to 15 °C and above (Fig. 1). Despite continued warming during April and May, emergence was moderate until June. A relatively close correspondence between temperature and emergence is evident for the spring cercariae, which vacated their hosts at about the same temperature threshold (15 °C) during each tidal cycle (Fig. 2B). As cool ocean water flooded the marsh, the shallow water mass was warmed first from contact with the mudflat and then by the sun. Cercarial emergence following snail inundation was typically delayed up to 2 h, until water temperature exceeded 15 °C.

During the warm summer months, June to September, when water temperature was above 18 °C (average of 21 °C), the number of emerged cercariae increased substantially (Fig. 1). Larvae left snails as soon as they were inundated (Fig. 2C). Despite similarity in temperature (1–2 °C difference) between paired day/night collections, few or no cercariae were collected at night (Table 2). Seawater cooled by about 3 °C in October, but emergence remained high until November. Once temperature dipped below 18 °C, only the cool-water species emerged.

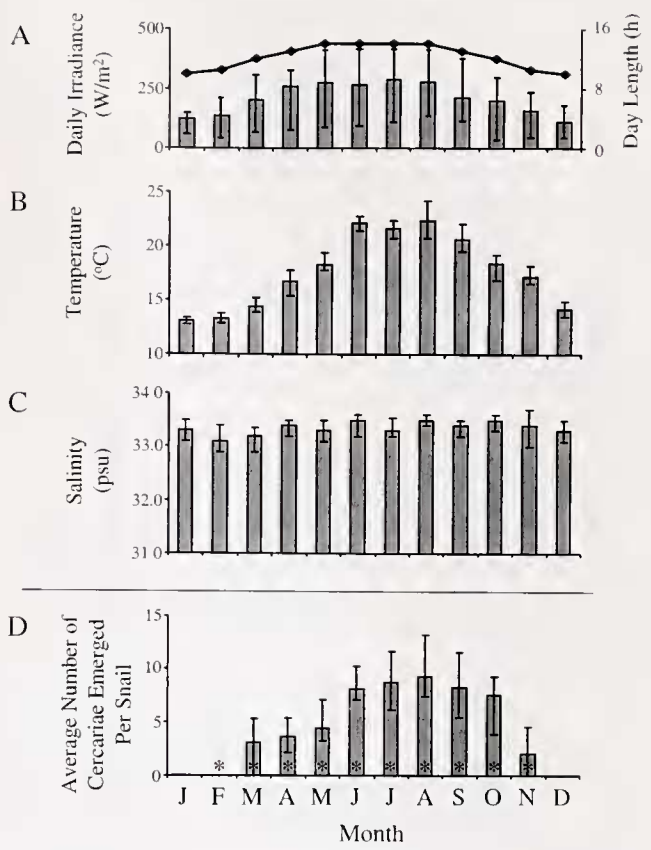

Figure 1. Variation throughout the year in average monthly (A) daily irradiance (histograms) and day length (diamonds), (B) water temperature, (C) salinity, (D) cercarial emergence and snail host presence on the mudflat (*). Histograms represent monthly means, and vertical bars plot the ranges. For day length, ranges are smaller than the data points. Irradiance data were taken from the California Irrigation Management System website (www.cimis.water.ca.gov) and day length from the United States Naval Observatory website (http://aa.usno.navy.mil).

Observations were made on the appearance of dominant species in the samples. *Himasthla rhigedana* (Dietz) and *Parorchis acanthus* (Nicoll) were the first species collected during the cool spring months (March to May), making up about 90% of the cercarial population. Other species, such as *Renicola buchanani* (Cohn), *Euhaplorchis californensis* (Martin), and *Microphallid* sp. (Martin), first appeared in late May or early June. Due to its high prevalence (Kuris, 1990) and fecundity, *E. californensis* numerically dominated the June to September samples. Together, *H. rhigedana* and *E. californensis* composed more than 75% of all cercariae collected throughout the day during the warm summer months.

Whereas cercarial emergence was high and relatively constant from June through September, duration of emergence events varied considerably among the 21 summer daylight samples. A stepwise multiple regression analysis of

Table 1

Stepwise multiple regression analysis of environmental cues as sources of variation in the number of emerged cercariae

Source	*F*-value	df	*P*	% of variation explained
Temperature	37.14	1/31	<0.0001	68.3
Total irradiance	0.05	1/31	0.82	<1.0
Salinity	0.34	1/31	0.56	<1.0

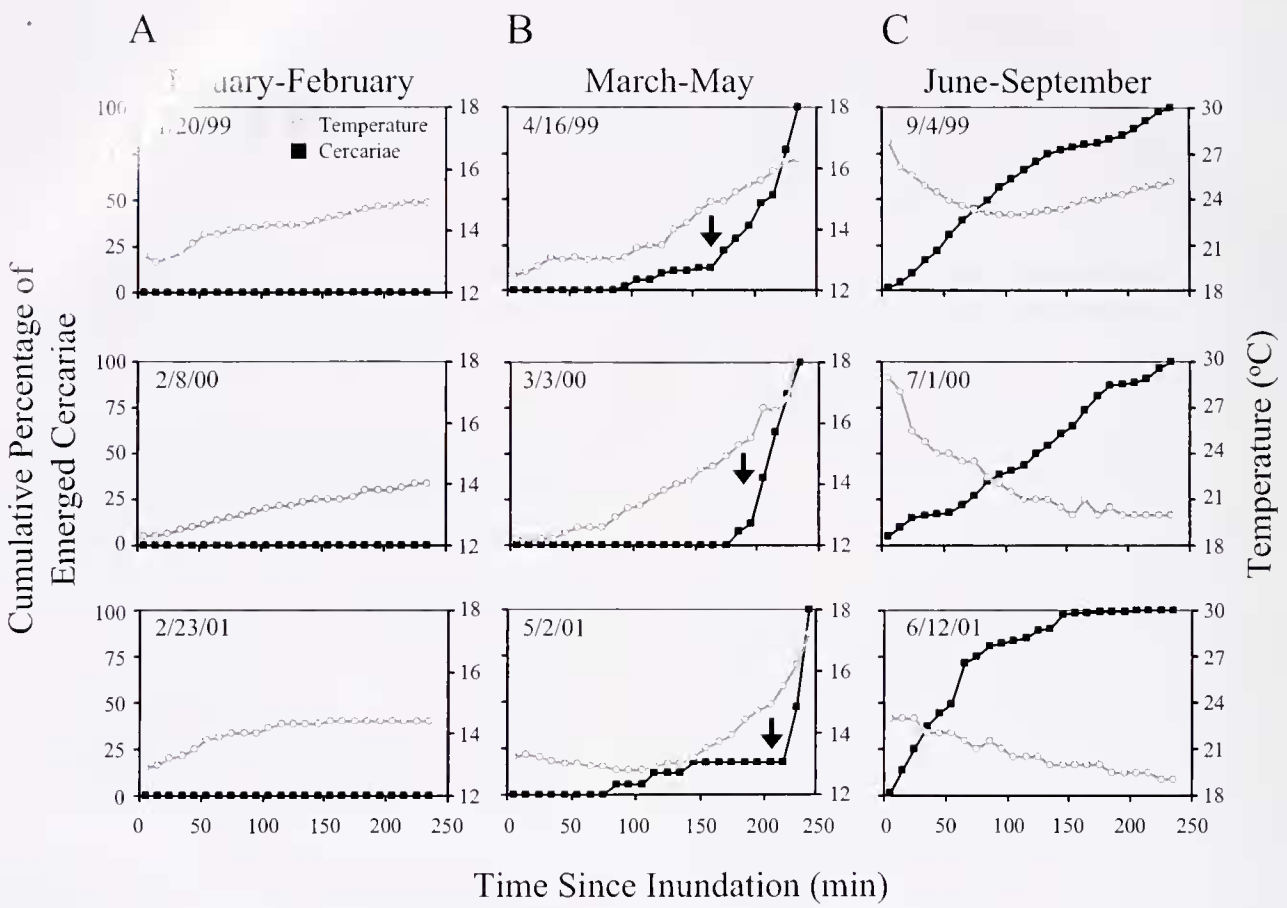

Figure 2. Three representative cercarial emergence patterns for each of three seasons of the year: (A) January–February (winter), (B) March–May (spring), and (C) June–September (summer). Water temperature (open circles) and cumulative percentages of emerged cercariae (closed squares) are from samples taken every 10 min. In (B), arrows correspond to the point in time when water temperature was first ≥ 15 °C.

emergence duration against temperature, salinity, time of year, and time of day yielded only one significant factor: time of day (Table 3). Duration of cercarial emergence decreased significantly throughout the day (Fig. 3A). Emergence intervals ranged from 4 h in the morning to 1 h or less by dusk (Figs. 3A and 4). This trend was present regardless of the numerical threshold for emergence duration (*i.e.*, 95%, as used here, *versus* 75% or 50%). Decreasing duration of cercarial emergence over the day resulted from an increase in the rate of emergence (number cercariae/h) (Fig. 3A, and least squares regression: $F = 27.36$, df = 1/19, $P < 0.0001$; $r^2 = 0.73$), not from a decrease in the total number of emerging cercariae (Fig. 3B, and $P = 0.68$). Long (~4 h) emergence durations during the early morning resulted from a relatively small but constant larval delivery over the entire period of inundation. As the day progressed, more cercariae emerged per unit time, but over a contracted interval. Approaching dusk, large numbers of cercariae emerged in a quick burst as inundation commenced.

Table 2

Number of cercariae shed during paired day/night collections

Date	Day or night	Average temperature (°C)	Number emerged per 4 h collection
1 Aug 2000	Day	21.4	1043
	Night	20.1	22
18 Aug 2000	Day	20.9	1543
	Night	19.9	11
24 July 2001	Day	22.3	1684
	Night	20.5	28
12 Aug 2002	Day	19.3	537
	Night	18.2	3
22 Aug 2002	Day	22.1	832
	Night	21.7	1
6 Sept 2002	Day	19.3	349
	Night	17.9	0

Water temperature is averaged over the entire 4-h collection. Mean light intensity during nighttime collections, even under full moon conditions, was always < 0.01 μmol/m²/s, compared to > 1035 μmol/m²/s during daylight collections.

Table 3

Stepwise multiple regression analysis of abiotic factors as sources of variation in emergence duration

Source	*F*-value	df	*P*	% of variation explained
Time of day	47.96	1/19	<0.0001	72.8
Time of year (month)	0.03	1/19	0.86	<1.0
Temperature	<0.01	1/19	0.95	<1.0
Salinity	1.78	1/19	0.19	8.1

Laboratory experiments

Host inundation. Throughout all laboratory studies, cercariae emerged only if snails were totally submerged. The average number of cercariae to emerge in paired treatments with submerged (3861 ± 599 SEM) and dry (0 ± 0 SEM) snails showed unequivocally that cercariae would not leave the host unless it was underwater (Student's *t* test: $t = 8.497$, df = 5, $P < 0.0001$). The muddy channel containing the highest snail densities was at a tidal height of about 1.3 m MLLW, so hosts spent 16 h/day out of water. Thus, inundation was a necessary condition for emergence.

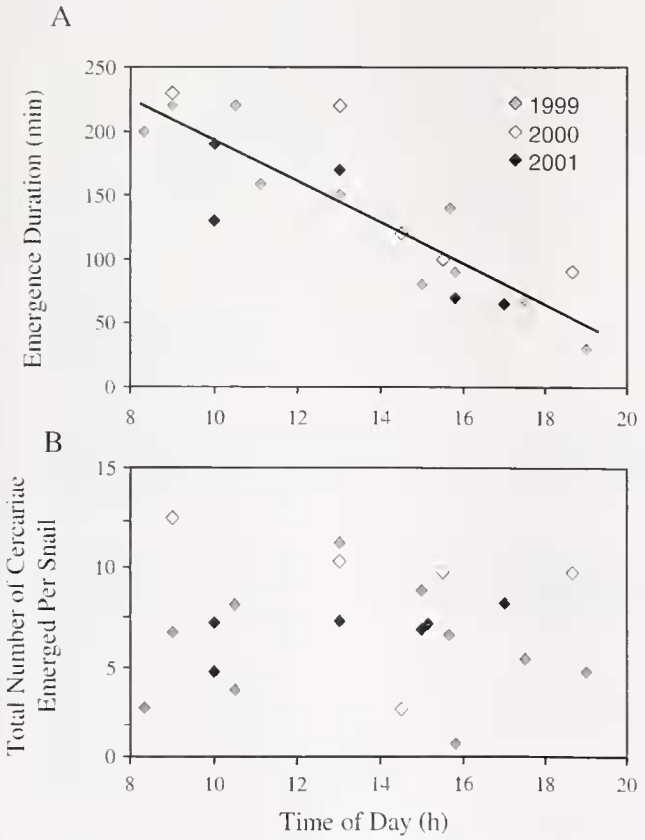

Figure 3. Effect of time of day (TOD) on cercarial emergence duration in the field. (A) The amount of time for 95% of all cercariae to emerge during a given event (4 h) as a function of TOD. (B) The total number of cercariae emerged per snail during a given event as a function of TOD.

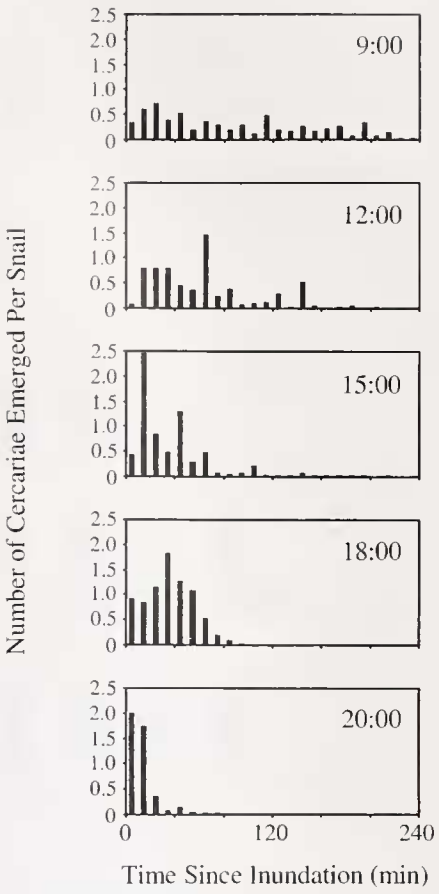

Figure 4. Representative series of histograms throughout the day, showing number of emerged cercariae per snail (10-min samples) as a function of time since tidal inundation. Times indicate when snail hosts were first submerged. Because snail inundation resulted from the natural high tide, each histogram was constructed on a different day.

Temperature. Cercariae of five trematode species emerged at three temperature thresholds (Fig. 5). Emergence of *H. rhigedana* and *P. acanthus* began in 15 °C water and continued for the maximal temperature (19 °C) tested. *Renicola buchanani* emerged at an intermediate threshold of 17 °C through 19 °C. The two warm-water species, *E. californensis* and *Microphallid* sp., vacated their hosts only in the warmest waters tested (18–19 °C). Two-way nested ANOVAs were performed for each species separately. The numbers of emerged cercariae were significantly different among temperatures ($P \leq 0.0001$ for all species) and individual snails ($P \leq 0.04$) for all species except *R. buchanani* ($P = 0.17$). Because the order (ascending, descending) of temperature change had no significant ($P \geq 0.11$) effect on the outcome, Scheffé *post hoc* comparisons were done for both series. Either way, there were two significantly ($P \leq 0.005$) different temperature groupings for all species: one within their emergence range and

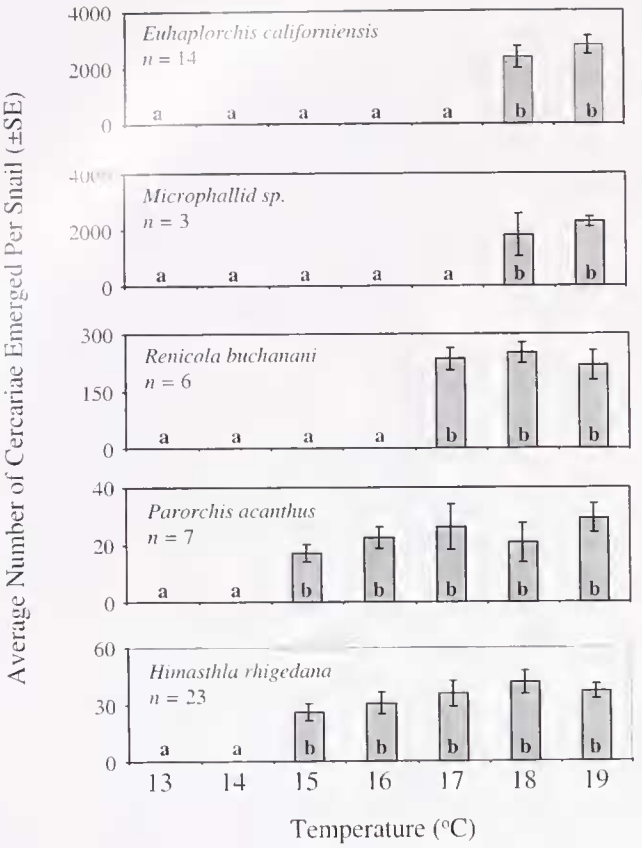

Figure 5. Number of cercariae emerged per snail over the temperature range 13–19 °C. Data are shown for five common trematode species. Histograms are means, and vertical bars are standard errors; n = number of snail hosts for a given trematode species. Means differ significantly when highlighted by a different letter (one-way ANOVA with *post hoc* Scheffé test: $P < 0.001$).

the other for the lower temperatures that prohibited emergence (Fig. 5).

Light. Although daylight is required for cercarial emergence (Table 2), even low intensities approximating those at dawn and dusk (40 μmol/m^2/s) were sufficient to trigger emergence under both field and laboratory settings. There was no significant difference, however, in the number of emerged cercariae or the duration of emergence at maximal midday intensity (2000 μmol/m^2/s) relative to a minimal dawn/dusk intensity (40 μmol/m^2/s) (t test: $t = 1.021$, df = 10, $P = 0.36$ for duration; $t = 0.475$, df = 10, $P = 0.65$ for number emerged).

Time of day. As in the field collections, the duration of cercarial emergence decreased over the course of the day (Fig. 6A), varying from a modest, protracted stream of cercariae at dawn to a contracted burst at dusk. There was a significant relationship between emergence duration and TOD (least squares regression: $F = 199.78$, df = 1/10, $P < 0.0001$; $r^2 = 0.93$), but not between number of emerged cercariae and TOD ($P = 0.28$) (Fig. 6B). Moreover, there was a noticeable change in the duration of emergence when the host light:dark cycle was shifted forward or back (Fig. 7). As predicted, emergence duration decreased when sunrise was shifted forward, implying that snails perceived a later time of day. Likewise, emergence duration increased when sunrise was shifted back. These changes agree qualitatively with the diel pattern observed in both the field and laboratory. Shifting the light:dark cycle had no apparent effect on the number of cercariae emerging (data not shown).

Discussion

All digenetic trematodes have a free-swimming cercarial stage that transmits infection from the first intermediate host to the second intermediate or definitive host. Given the short life span of cercariae, emergence timing may be optimized to enhance the probability of successful transmission. In this study, larvae emerged from *Cerithidea californica* over species-specific temperature ranges, exclusively during day-

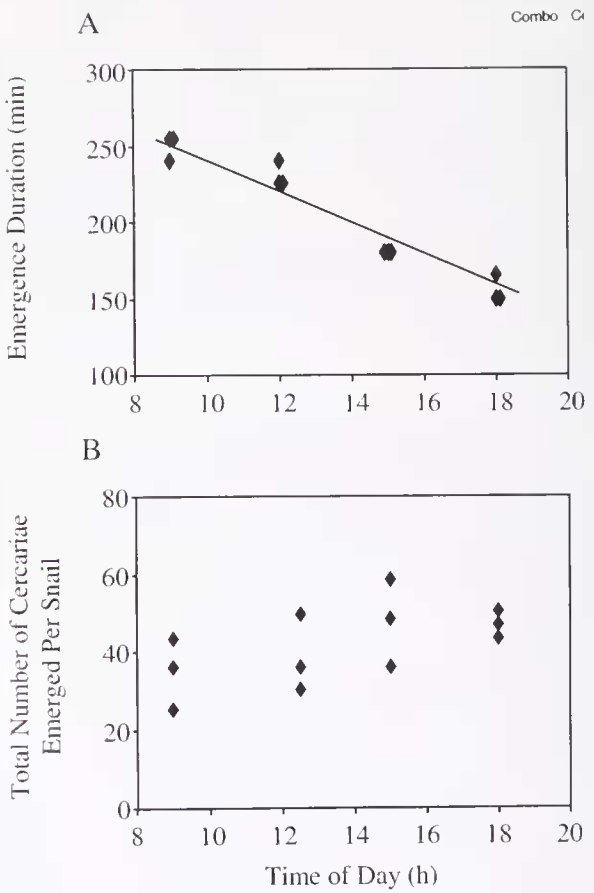

Figure 6. Effect of time of day (TOD) on emergence duration in the laboratory. (A) The amount of time for 95% of all cercariae to emerge during a given event (4 h) as a function of TOD. (B) The total number of cercariae emerged per snail during a given event as a function of TOD.

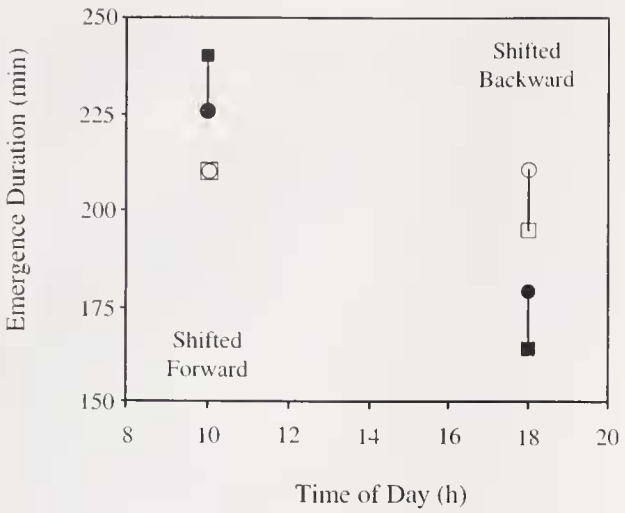

Figure 7. Effect on emergence duration of shifting sunrise forward or backward 8 h, compared to unshifted control groups. Filled symbols indicate control values by year (2000 ●, 2001 ■), and open symbols indicate shifted regimes (2000 ○, 2001 □).

light hours, and only when snails were submerged (Fig. 8). The three determinants operated over different time scales: temperature monthly, light diurnally (24-h period), and water depth tidally (12-h period). Light/dark and tidal cycles also varied daily, according to the solar and lunar cycles, respectively. Each stimulus creates a necessary condition for the next, forming a hierarchy of environmental cues. Duration of cercarial emergence varied over the day and was under endogenous control. Although many studies have identified two or more factors that affect propagule release, evidence for a cue hierarchy is relatively rare (some exceptions include Levy *et al.*, 2000; Watson *et al.*, 2000).

The timing and length of the transmission season was controlled, at least in part, by water temperature. Host snails (*C. californica*) occupied the muddy surface of the channel when water was 12 °C or above. Thus, the much narrower temperature ranges for emergence of the five trematode species may forecast the presence of second intermediate hosts. For example, cercariae of *Himasthla rhigedana* and *Parorchis acanthus* emerged over the broadest temperature range (≥ 15 °C) and use the eurythermal *C. californica* as a second intermediate host. In contrast, *Euhaplorchis californiensis*, which emerged only in ≥ 18 °C water, infects several fish species that increase in abundance over the warming summer (Fritz, 1975; Brooks, 1999; Madon *et al.*, 2001).

Temperature thresholds for cercarial emergence occur in both estuarine and freshwater habitats (Lo and Lee, 1996; McCarthy, 1999). The number of emerged cercariae also varies with temperature in freshwater species (Shostak and Esch, 1990; Lyholt and Buchmann, 1996). Temperature-dependent emergence in freshwater species has been ascribed to a trade-off between the number emerged (positively related to temperature) and functional (infective), and the total life span (both negatively related to temperature) (McCarthy, 1999; Toledo *et al.*, 1999).

A characteristic common to estuarine and freshwater systems is diel variation in cercarial emergence, supporting our first hypothesis (see *Introduction*). In our study, cercariae

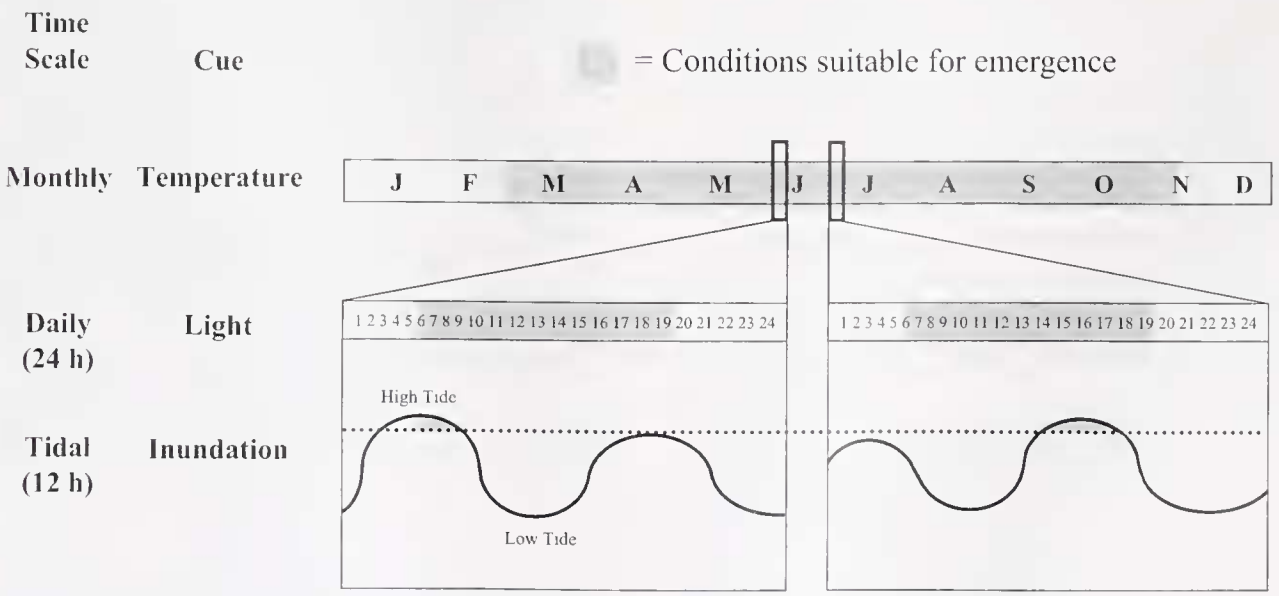

Figure 8. Diagram of the hierarchy of cues controlling cercarial emergence at monthly (temperature), daily (light), and tidal (host inundation) time scales. Dotted line represents tidal height (1.3 m above MLLW) of mudflat where sampling took place.

left submerged snails only during daylight hours. In intertidal estuaries, tidal currents are a predictable signal that determines both submersion time of the hosts and aquatic transport of the larvae. Thus, emergence of cercariae tracked with the daytime flood tide, which changes daily according to the lunar cycle. The daylight requirement may indicate that light is a critical cue. In laboratory flume studies, photo-triggered downward swimming of *H. rhigedana* was effective in slow flows typical of CSMR (Fingerut *et al.*, 2003). This activity quickly brings larvae to the bed for contact with benthic hosts. Moreover, we observed that *E. californiensis* cercariae swam upward in response to light (authors' unpubl. data). Such behavior could increase contact with host fish in the water column. Likewise, most freshwater cercariae are shed on the bottom, and light or gravity directs swimming up or down, depending on the location of the next host (Combes *et al.*, 1994). Within the CSMR estuary, cercarial emergence during flood tides would transport larvae 100–300 m/h (Fingerut, 2003). Yet, emerged *H. rhigedana* were dispersed only about 1–2 m before encystment (Fingerut, 2003). Directed swimming by larvae thus may have greatly shortened their transport distances.

Abbreviated dispersal would retain asexually reproduced larvae within the marsh, where local mixing could diversify the gene pool of cercariae encysting on subsequent hosts. All except 2 of 18 species within the southern California estuarine trematode guild have benthic second intermediate hosts (snails and crabs) that are interspersed within first intermediate host populations. In fact, some trematodes (*e.g., H. rhigedana, P. acanthus*) use the same first and second host species. Turbulent mixing could commingle larval genotypes throughout the marsh, enhancing genetic diversity of the multiple cercariae that encyst each second host. Ensuing sexual reproduction between dissimilar genetic parasites within the definitive shorebird host may enhance fitness of the trematode population (*e.g.,* Scheltema, 1971; Jablonski, 1986; Pechenik, 1999, for marine invertebrates).

As in digenetic trematodes, larval release in free-living estuarine invertebrates is contingent on tidal flows. For example, estuarine mud-dwelling isopods (*e.g., Paragnathia formica* [Hess]) release larvae only when high tides reach their burrows, facilitating transport (Tinsley and Reilly, 2002). Shore crabs living in high-intertidal refuges limit larval release to nighttime spring tides (Morgan and Christy, 1995; Hovel and Morgan, 1997). Darkness reduces predation on spawners, whereas large-amplitude tides flush larvae away from diurnal predators. Similarly, in tidally influenced riverine habitats, adult terrestrial crabs, such as *Sesarma haematocheir* (de Haan), release larvae on a nighttime semilunar cycle that minimizes predation on adults while providing optimal conditions for survival and dispersal of larvae (Saigusa, 1982).

Endogenously regulated emergence differs between the estuarine intertidal trematodes studied here and many freshwater species. This result was not predicted by our second hypothesis (see *Introduction*), and may be explained by the major synchronizing agents in these systems. In freshwater, emergence timing is often under endogenous control and is directly linked to presence of the subsequent host. For freshwater schistosomes, definitive vertebrate hosts frequent the waterfront on a predictable innate cycle. Circadian rhythms of cercarial emergence are tuned to the biological clocks and activity patterns of these vertebrates (Combes, 1991; Combes *et al.*, 1994). For example, maximal emergence of some schistosome cercariae corresponds with the proximity of their bovine hosts. Cercariae emerge and swim to the surface in the morning, thus infecting the animals while they drink at the waterfront (Mouahid *et al.*, 1991; Raymond and Probert, 1991). Likewise, afternoon emergence peaks occur in trematode species infecting human hosts that wash, play, or drink at midday (Theron, 1984; Pages and Theron, 1990). Emergence in non-schistosome species, such as *Proterometra edneyi* (Uglem) also occurs on a circadian cycle timed to the presence of fish that are their second intermediate hosts (Lewis *et al.*, 1989). There is no emergence rhythm in cercariae of *Fasciola hepatica* (Linnaeus), however, because second intermediate host plants are always available (Bouix-Busson *et al.*, 1985), making synchronization unnecessary.

In the CSMR intertidal estuary, duration rather than timing of emergence was apparently under endogenous control, and the tide was the synchronizing agent. There is a finite, tidally determined period of sufficient immersion for larval transport in lighted hours. Emergence duration decreased throughout the day in response to dwindling daylight. An endogenous rhythm associated with the light:dark cycle may be responsible for the changing emergence rate over the course of a suitable flood tide. Apportioning larvae over the entire lighted submerged interval maximizes the dispersal envelope. Contraction of emergence duration throughout the day may optimize use of remaining daylight hours. As the day progresses, larger pulses of larvae must emerge over a shorter interval to take advantage of the vestigial light. Such diel adjustments to the emergence period are unnecessary in freshwater systems, where water depth is relatively stable. Thus driven by the submergence constraints of their natal habitat, the emergence strategies of estuarine cercariae involve both exogenous and endogenous factors that optimize transport to tidally accessible hosts.

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